CD8+ T Cell Abnormalities in Scleroderma Lung Disease

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Summary

These data are consistent an important role for CD8+ T cells in the complex pathology of lung fibrosis in scleroderma. These cells are increased in number, are activated, appear to have undergone initial selection by antigen stimulation, with increased oligoclonality, and have an unusual phenotype for CD8+ T cells, that of production of type 2 cytokines. Cytolytic pathways appear to be engaged. These CD8+ T cells may persist in vivo because of reduced activation-induced cell death. The cytokines and growth factors that they produce may directly stimulate extracellular matrix production.

Introduction

In animal models, the pathology of progressive pulmonary fibrosis includes epithelial cell injury and alveolar inflammation, organization of the resultant alveolar exudate, and incorporation of the alveolar fibroproliferative process into alveolar walls. Each stage needs to be intact for lung fibrosis to occur. T cells are essential in the development of lung fibrosis in certain animal models, mediated in part through cytotoxic effects, cytokine and growth factor production, and regulation of activation of alveolar macrophages. The role of T cells in the development of lung fibrosis in scleroderma remains unknown.

Methods

Bronchoalveolar lavage (BAL) fluids and cells were obtained from patients with scleroderma. Flow cytometry was used to assess T cell subsets in unfractionated BAL cells. CD8+ T cells were isolated by magnetic bead selection. DNA arrays were used to assess global gene expression, PCR was used to assess diversity of T cell receptor (TCR) gene expression and cytokine mRNA levels, and ELISA was used to assess protein levels in BAL fluids.

Results

Total numbers of activated (HLA-DR+, IL-2R+) CD8+ T cells were increased two- to three-fold in scleroderma patients with lung inflammation by BAL cell differential, compared to patients without lung inflammation. The diversity of T cell receptor junctional region lengths expressed in these CD8+ T cells was more restricted than in controls, indicating increased oligoclonality of these CD8+ T cells. This finding was confirmed by sequence analyses of TCR junctional region DNAs. DNA array analyses showed increased expression of genes associated with T cell activation, Th2 cells, and reduced activation-induced cell death. They also demonstrated increased expression of mRNAs for the profibrotic growth factor oncostatin M, integrin b6, which activates transforming growth factor b, and membrane-type matrix metalloproteinases 1 and 2. The expression of a Th2 phenotype was consistent with PCR data showing increased production of interleukin-4 (IL-4) and IL-5 mRNAs in CD8+ T cells. Increased expression of oncostatin M was confirmed by ELISA of BAL fluids. In addition, granzyme A and granzyme B levels were increased in BAL fluids from patients with lung inflammation, suggesting activation of cytolytic pathways *in vivo* in these patients. In small numbers of patients, production of Th2 cytokine mRNAs was associated with progressive restrictive lung disease. In a larger number of patients, multivariate analyses indicated that higher numbers of activated CD8+ T cells in BAL fluids were associated with subsequent decline in forced vital capacity and diffusing capacity for carbon monoxide.